EMERGING PATHOGENS

Although many of the established waterborne pathogens have been controlled by sanitation measures and water treatment processes, new diseases continue to be identified, and new discoveries present a better understanding of existing chronic diseases. Many of these discoveries raise questions about possible waterborne transmission. The bacteria that now need to be considered in this developing area are *Helicobacter pylori*, *Mycobacterium* species, *Burkholderia pseudomallei* and *Francisella tularensis*.

Helicobacter pylori

Although spiral-shaped organisms have been observed in the stomachs of humans for many years, it was not until 1982 that a *Campylobacter*-like organism was isolated from patients with gastritis and a causative relationship between a new species, *Helicobacter pylori*, and gastric disease realized. *H. pylori* is a pathogen of global proportions and is generally accepted as the cause of most gastric and peptic ulcers. These diseases may lead to gastric adenocarcinoma.

H. pylori occurs worldwide in developing and developed countries. Where low degrees of hygiene and socioeconomic problems exist, infection rates may approach 100%. In developed countries, infection rates are probably between 30 and 60%.

Transmission from person to person is not fully understood, mainly because of the difficulty in culturing the organism and identifying it outside the body. Epidemiological studies show the cluster phenomenon of *H. pylori* infection in families. It is suggested that infected mothers may play a key role in transmission within families.

H. pylori has been identified in faeces, and it is assumed that transmission is therefore oral–oral or faecal–oral. The organism has not been isolated from the environment or from drinking water, and waterborne transmission remains a possibility that should be investigated. The epidemiology, however, points to person-to-person transmission in early life.

Burkholderia pseudomallei

Burkholderia pseudomallei is the cause of melioidosis, an acute pneumonia often followed by systemic infection with later presentations of abscesses. The organism is widespread in the environment and was originally described in Rangoon in patients compromised by severe poverty who had presumably inhaled the organism in dust when sleeping on the ground. It occurs commonly in southeast Asia and has been detected in service personnel repatriated from those areas in the past. It was also investigated as a biological weapon by several nations, to be released as an aerosol and cause pneumonia infection in those exposed.

Francisella tularensis

Tularaemia is a zoonosis caused by a highly infective and virulent organism *Francisella tularensis*.

It is transmitted to humans who come in close contact with the animal reservoir, arthropods that feed on them or debris and dust associated with them.

It can also be transmitted through the ingestion of contaminated water. Human epidemics sometimes occur and are associated with epizootics in the animal populations, evidenced by die-offs. There are several presentations of tularaemia in humans, depending on the route of exposure. Ingestion usually results in oropharyngeal tularaemia, with fever, pharyngitis and cervical lymphadenitis.

Following the recent war in Kosovo, over 900 suspected cases of tularaemia were identified and 327 cases confirmed serologically. The epidemiological investigation pointed to rodent-contaminated wells, and rodent carcasses found in some wells tested positive for *F. tularensis*.

In a waterborne outbreak reported from Spain, 19 cases who had contact with river-caught crayfish were identified. Attempts to isolate *F. tularensis* from water were unsuccessful. Drinking-water was not involved. *F. tularensis* is notoriously difficult to culture, requiring a source of cysteine. *F. tularensis* was investigated and developed as a biological weapon; the infectious dose was found to be extremely low — 10 organisms.

BIOTERRORISM THREAT AGENTS

The classical biological warfare agents that were investigated and sometimes developed by certain countries in the past were intended for aerosol dissemination to cause infection in those exposed. Since the events of 11 September 2001 in the USA and the anthrax letters, awareness of the threat of bioterrorism has been raised considerably. As the consequences could be disastrous, much planning and international cooperation have occurred to prevent any future deliberate releases or to limit their effects, should they occur.

BACILLUS SPECIES

gram-positive, form endospores, and are either strict aerobes or aerotolerant anaerobes (that is, they can grow in the presence of oxygen, but do not require it).

Most of the species of Bacillus are found in soil and water and are usually encountered in the medical laboratory as airborne contaminants.

B. anthracis, the cause of the disease anthrax, is clinically the most important member of this genus.

A. Bacillus anthracis

- Form very long chains of rods and in culture is nonmotile and nonhemolytic colonies are characterized by a rough, uneven surface with multiple curled extensions at the edge resembling a "**Medusa head**."
- *B. anthracis* has a D-glutamic acid polypeptide capsule of a single antigenic type that has antiphagocytic properties.
- The organism is also is a potent producer of one or more exotoxins, which although they have been given multiple names (lethal factor, edema factor, protective antigen), represent separate activities of a protein complex. In various combinations and configurations these proteins may exhibit binding, cytolytic, or enzymatic activity.

Clinical significance

a. Cutaneous anthrax:

Upon introduction of organisms or spores that germinate, a papule develops. It rapidly evolves into a painless, black, severely swollen "malignant pustule,"

b. Pulmonary anthrax (**Woolsorter's disease**)**:** Caused by inhalation of spores

c. Enteric anthrax: A severe form of gastroenteritis with fever, abdominal pain and bloody diarrhoea, due to ingesting infected meat. Septicaemia often develops.

d. Meningoencephalitis: Usually as a complication of septicaemia and occasionally as primary anthrax meningoencephalitis.

DIAGNOSIS

Caution: *B. anthracis* is a high risk infectious pathogen, therefore handle specimens and infected material with care, wearing protective gloves and face mask, and following recommended safety procedures. **Use 4% v/v formalin** solution to decontaminate infected material and laboratory ware.

Sample: skin lesions, sputum, blood, and CSF are the primary means of anthrax diagnosis.

Morphology

Gram positive (or Gram variable) non-motile bacillus, often appearing joined end to end in chains.

In smears from specimens: Bacilli are capsulated. The capsular material often appears irregular and fragmented. When stained using Loeffler's polychrome (McFadyean) methylene blue, the bacilli stain blue and the capsular material stains purple-pink. Alternatively, **Giesma stain** can also be used when MacFadyean methylene blue is not available.

In smears from aerobic cultures: Bacilli are non-capsulated but contain oval spores (same diameter as the bacilli), giving the organisms a beaded appearance.

Fixation of smears

B. anthracis is not killed by heat-fixation. Smears should be chemically fixed by immersing the dry smears in a container of **potassium permanganate** 40 g/l solution for 10–15 minutes.
Important: When organisms resembling *B. anthracis* are seen in smears, specimens should be sent for further testing to the nearest Public Health Laboratory and public health officials notified as soon as possible

Culture
B. anthracis grows aerobically and anaerobically (facultative anaerobe). The temperature range for growth is 12–45 ºC with an optimum of 35–37 ºC. Spore formation is best between the range 25–30 ºC.

Blood agar: *B. anthracis* produces large 2–5 mm in diameter, grey-white, irregular colonies with wavy edges. The colonies are nonhaemolytic or only slightly haemolytic. The saprophytic species are usually β-hemolytic and motile; these features can be used to exclude *B. anthracis.*
Gelatin stab culture
The organism slowly liquefies the gelatin.
Prevention
Pasteur' s vaccine used a live strain attenuated by repeated subculture that resulted in the loss of a plasmid encoding toxin production.

Hospital acquired Infections

'Nosocomial' or 'healthcare associated infections' (HCAI) appear in a patient under medical care in the hospital or other health care facility which was absent at the time of admission. These infections can occur during healthcare delivery for other diseases and even after the discharge of the patients. Additionally, they comprise occupational infections among the medical staff. Invasive devices such as catheters and ventilators employed in modern health care are associated to these infections. Of every hundred hospitalized patients, seven in developed and ten in developing countries can acquire one of the healthcare associated infections. Populations at stake are patients in Intensive Care Units (ICUs), burn units, undergoing organ transplant and neonates.

Types of nosocomial infections

The most frequent types of infections include central line associated bloodstream infections, catheter-associated urinary tract infections, surgical site infections and ventilator-associated pneumonia.

Central line-associated bloodstream infections (CLABSI): CLABSIs are deadly nosocomial infections with the death incidence rate of 12%–25%. Catheters are placed in central line to provide fluid and medicines but prolonged use can cause serious bloodstream infections resulting in compromised health and increase in care cost.

Catheter associated urinary tract infections (CAUTI)

CAUTI is the most usual type of nosocomial infection globally. According to acute care hospital stats in 2011, UTIs account for more than 12% of reported infections. CAUTIs are caused by endogenous native microflora of the patients. Catheters placed inside serves as a conduit for entry of bacteria whereas the imperfect drainage from catheter retains some volume of urine in the bladder providing stability to bacterial residence. CAUTI can develop to complications such as, epididymitis and prostatitis in males, and pyelonephritis, cystitis and meningitis in all patients.

Surgical site infections (SSI)

SSIs are nosocomial infections be fall in 2%–5% of patients subjected to surgery. These are the second most common type of nosocomial infections mainly caused by Staphylococcus aureus resulting in prolonged hospitalization and risk of death. The pathogens causing SSI arise from endogenous microflora of the patient. The incidence may be as high as 20% depending upon procedure and surveillance criteria used.

Ventilator associated pneumonia (VAP)

VAP is nosocomial pneumonia found in 9–27% of patients on mechanically assisted ventilator. It usually occurs within 48 h after tracheal incutation. 86% of nosocomial pneumonia is associated with ventilation. Fever, leucopenia, and bronchial sounds are common symptoms of VAP

Nosocomial pathogens: Pathogens responsible for nosocomial infections are bacteria, viruses and fungal parasites. These microorganisms vary depending upon different patient populations, medical facilities and even difference in the environment in which the care is given.

Bacteria: are the most common pathogens responsible for nosocomial infections. Some belong to natural flora of the patient and cause infection only when the immune system of the patient becomes prone to infections.

Acinetobacter is the genre of pathogenic bacteria responsible for infections occurring in ICUs. It is embedded in soil and water and accounts for 80% of reported infections. Bacteroides fragilis is a commensal bacteria found in intestinal tract and colon. It causes infections when combined with other bacteria. Clostridium difficile cause inflammation of colon leading to antibiotic-associated diarrhea and colitis, mainly due to elimination of beneficial bacteria with that of pathogenic. C.difficile is transmitted from an infected patient to others through healthcare staff via improper cleansed hands. Enterobacteriaceae (carbapenem resistance) cause infections if travel to other body parts from gut; where it is usually found. Enterobacteriaceae constitute Klebsiella species and Escherichia coli. Their high resistance towards carbapenem causes the defense against them more difficult. Methicillin-resistant S. aureus (MRSA) transmit through direct contact, open wounds and contaminated hands. It causes sepsis, pneumonia and SSI by travelling from organs or bloodstream. It is highly resistant towards antibiotics called beta-lactams

Epidemiology of nosocomial infections Nosocomial infection affects huge number of patients globally, elevating mortality rate and financial losses significantly. According to estimate reported of WHO, approximately 15% of all hospitalized patients suffer from these infections.

Determinants

Risk factors determining nosocomial infections depends upon the environment in which care is delivered, the susceptibility and condition of the patient, and the lack of awareness of such prevailing infections among staff and health care providers.

1. Environment Poor hygienic conditions and inadequate waste disposal from health care settings.
2. Susceptibility: Immunosupression in the patients, prolonged stay in intensive care unit, and prolonged use of antibiotics.
3. Unawareness Improper use of injection techniques, poor knowledge of basic infection control measures, inappropriate use of invasive devices (catheters) and lack of control policies. In low income countries these risk factors are associated with poverty, lack of financial support, understaffed health care settings and inadequate supply of equipments

Reservoirs and transmission

1. Microflora of patient: Bacteria belonging to the endogenous flora of the patient can cause infections if they are transferred to tissue wound or surgical site. Gram negative bacteria in the digestive tract cause SSI after abdominal surgery.
2. Patient and staff: Transmission of pathogens during the treatment through direct contacts with the patients (hands, saliva, other body fluids etc.) and by the staff through direct contact or other environmental sources (water, food, other body fluids).
3. Pathogens living in the healthcare environment i.e. water, food, and equipments can be a source of transmission. Transmission to other patient makes one more reservoir for uninfected patient

Prevention of nosocomial infection:

Being a significant cause of illness and death, nosocomial infections need to be prevented from the base line so that their spread can be controlled.

Transmission from environment: Unhygienic environment serves as the best source for the pathogenic organism to prevail. Air, water and food can get contaminated and transmitted to the patients under healthcare delivery. There must be policies to ensure the cleaning and use of cleaning agents on walls, floor, windows, beds, baths, toilets and other medical devices. Proper ventilated and fresh filtered air can eliminate airborne bacterial contamination. Regular check of filters and ventilation systems of general wards, operating theatres and ICUs must be maintained and documented. Infections attributed to water are due to failure of healthcare institutions to meet the standard criteria. Microbiological monitoring methods should be used for water analysis. Infected patients must be given separate baths. Improper food handling may cause food borne infections. The area should be cleaned and the quality of food should meet standard criteria

Transmission from staff: Infections can be transferred from healthcare staff. It is the duty of healthcare professionals to take role in infection control. Personal hygiene is necessary for everyone so staff should maintain it. Hand decontamination is required with proper hand disinfectants after being in contact with infected patients. Safe injection practices and sterilized equipments should be used. Use of masks, gloves, head covers or a proper uniform is essential for healthcare delivery

Hospital waste management Waste from hospitals can act as a potential reservoir for pathogens that needs proper handling. 10–25% of the waste generated by healthcare facility is termed as hazardous. Infectious healthcare waste should be stored in the area with restricted approach. Waste containing high content of heavy metals and waste from surgeries, infected individuals, contaminated with blood and sputum and that of diagnostic laboratories must be disposed off separately. Healthcare staff and cleaners should be informed about hazards of the waste and it's proper management

Control of nosocomial infections: Despite of significant efforts made to prevent nosocomial infections, there is more work required to control these infections. An efficient infection control program is shown in Figure.